Anti-bacterial activity of *Moringa spp* seed extracts and their water flocculation ability

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ABSTRACT

Water-borne diseases are some of the main problems in developing countries; about 1.6 million people are compelled to use contaminated water. In many communities in the Winam Division, water clarification/purification methods are often inappropriate because of the high cost and low availability of chemical coagulants, thus they consume water contaminated with disease causing microorganisms leading to outbreak diseases like typhoid. Natural products from *Moringa spp.* have been used as primary coagulants for water treatment. Objectives of the research were to establish the anti-bacterial activity of *M. oleifera* and *M. stenopetala* and their water clarification ability. 1 kg powder of shade dried and milled seeds of the *M. oleifera* and *M. stenopetala* were serially soaked in 2 litres of n-hexane and 2 litres of methanol to extract the active components and placed in a shaker for 4 hrs. Samples of water collected were processed for water coagulation studies. The n-hexane and methanol filtrates of *M. oleifera* Lam and *M. stenopetala* seed extracts were then concentrated in a round-bottomed flask using a rotary evaporator under low temperature to obtain the extracts. 340ml n-hexane extract, 126ml methanol extract of *M. oleifera* and 226ml n-hexane extract, 67ml methanol extract of *M. stenopetala* were obtained. The anti-bacterial activity of n-hexane and methanol extracts of *M. oleifera* and *M. stenopetala* against *Salmonella typhii, Vibrio cholera* and *Escherichia coli* and water clarification ability of their methanol extracts were then evaluated using randomized complete block design. The results were analyzed using Analysis of Variance. The results indicated that, n-hexane extract had a higher inhibition on *Salmonella typhii* than *Vibrio cholera* and *Escherichia coli* and *M. oleifera* was better water clarifier than *M. stenopetala*. Aluminium sulphate achieved lower turbidity than *M. oleifera* and *M. stenopetala* extracts. The results showed that *M. oleifera* and *M. stenopetala* had antibacterial and water clarification ability. The outputs from this research would generate baseline data prerequisite for the future development of water purification and clarification products.

CHAPTER ONE

INTRODUCTION

1.1 Background

Water is essential for life. Both plants and animals need it. Around 1.1 billion people in poor countries do not have access to clean water sources (UNDP, 2003) and diarrheal diseases cause an estimated 2.2 million deaths a year (WHO, 1999). Unfortunately, communities where diarrhea is a leading cause of morbidity and mortality often lack the capacity and the resources to establish and sustain centrally purified water free from sewage (WHO, 1999). Contamination of water during collection, transportation, and storage at home presents a serious health risk to millions of households in developing countries. Several studies have shown an increased risk of diarrhea because of inadequate water storage (Mintz *et al.*, 1995). Regardless of where or how the water is collected, storage vessels with wide openings such as pots or buckets are easily contaminated with feaces, through the introduction of cups, dippers, or hands. Flies, cockroaches, and rodents may also contaminate water. The importance of household based treatment of water at point of use in reducing diarrheal disease has been increasingly recognized (Mintz *et al.*, 1995; Mintz *et al.*, 2001; Clasen and Cairncross, 2004).

Several organizations have resorted to purification of water at the point of use by applying simple household bleach (sodium hypochlorite) to disinfect the water (Mintz *et al.*, 2001), they use narrow mouthed storage vessels to store the clarified water and educate the people about the causes and prevention of diarrhea (Clasen and Cairncross, 2004). Several studies in developing countries have shown that household based disinfection of drinking water with sodium hypochlorite or with a new flocculants-disinfectant reduces the incidence of diarrhea by 20-48% (Semenza *et al.*, 1998; Quick *et al.* 1999; Quick *et al.*, 2002; Reller *et al.*, 2003). A key challenge has been to discourage people

from using disinfectants that may adversely affect the taste of drinking water and may not as well improve its appearance. Furthermore, sodium hypochlorite, a widely used household based disinfectant, is less effective in highly turbid water (Crump *et al.*, 2004) and for pathogens resistant to chlorine such as *Cyclospora cayetanensis* or *Cryptosporidium parvum* (Meinhardt *et al.*, 1996).

Until recently, interventions to improve the safety of water and sanitation have focused on safe disposal of excreta and proper use of water for personal hygiene rather than on paying attention to water quality (Esrey and Habicht, 1986). A recent review by the World Health Organization found that low cost simple and acceptable interventions in households can improve the biological quality of water stored in the home and hence reduce the risk of diarrhea and death (WHO, 1999). Such interventions include boiling, chlorination, and coagulation-flocculation of water. Unfortunately, boiling consumes a lot of energy (it takes 1 kg of wood to boil 1 litre of water) and the cost may be prohibitive particularly in the developing countries where wood and other biomass fuels are not always available (Tumwine, 2005). Moreover, burning wood can lead to deforestation with serious environmental degradation (Sobsey, 2005).

Other intervention strategies such as use of cloth have been found to remove zooplankton and phytoplankton carrying *Vibrio cholerae* (Huq and Xu, 1996) and are used extensively for the eradication of guinea worm. However, cloth is not recommended for routine filtration of water in the home because its pores are too large to remove bacteria and viruses. Chemical coagulants such as alum (aluminium sulphate), lime, ferric chloride and synthetic polymers are also available in the market for precipitation of particles and microbes. They can be used in households to reduce transmission of diarrheal disease but their use in developing countries has been limited by issues of access, effectiveness, cost, and sustainability (Reller *et al.*, 2003). This study was carried out in

Winam Division, Kisumu District where residents use surface water obtained from ponds, rivers, springs, wells and Lakes for drinking. The water is usually contaminated with both human and animal feaces. The only common water clarification method used by the residents is modest turbidity reduction by settling, decanting, cloth filtration and clarification with aluminium sulphate (Makutsa *et al.*, 2001). This study was intended to establish the antibacterial activity of extracts of *Moringa oleifera* and *M. stenopetala* and compare their water clarification ability with aluminium sulphate.

1.2 Problem statement

River water taken for household use can be full of suspended matter, particularly in the rainy season. The water carries silt particles, solids, bacteria and other micro-organisms (some of which cause disease). It is very important to remove as much as possible of these materials before people use the water. Many microorganisms contaminating water can cause gastroenteritis or inflammation of the stomach and intestinal lining. These include Shigellosis caused by *Shigella sonnei*, Salmonellosis caused by *Salmonella gastroenteritis*, Cholera caused by *Vibrio cholerae* and typhoid fever caused by *Salmonella typhii*. Conventional water purifiers such as aluminium sulphate and chlorine have been used, but due to high cost and unavailability, households within Winam Division use unpurified water leading to increased cases of the water borne disease outbreaks (Ouma *et al.*, 2005). *Moringa spp* preparations can be used as a cheaper alternative to the conventional purifiers and disinfectants, however, their purification and disinfectant efficacies on the above microorganisms are not known. There is need therefore to establish the water purification and disinfectant efficacies of the *Moringa spp* for recommendation as an alternative to aluminium sulphate and chlorine in water purification.

1.3 Justification

Since over 80% of the Kenyan population live in the rural areas where water purification is rarely practiced due to high cost and unavailability of conventional purifiers and disinfectants, recurrent diseases like cholera and typhoid are unavoidable from among members of the communities living there. Because cholera and typhoid are prevalent in the Winam Division (Ouma *et al.*, 2005), there is need for water purification by the residence before being consumed. The population in the urban area is relatively safer from the diseases since the Kisumu City Council supplies them with purified water which is safe for consumption, however, the rural population use surface water contaminated with both human and animal feaces (Makutsa *et al.*, 2001). Chemical control measures have been in use for many years but consumers are now becoming more and more particular about them, not only because of cost factors but also the resultant quality, safety and reliability of products. Even in the international market, there is the European Retailers Good Agricultural Practices (EureGAP) protocol whose guidelines aim at producing products that are safe, environmental friendly, socially acceptable and of high quality. Few people in the rural areas of Winam Division use certain chemical preparations such as aluminium sulphate (Makutsa *et al.*, 2001) to purify water.

The use of aluminium sulphate has its setbacks. Several studies have reported that certain unspecified amount of this salt is soluble in water and when consumed, its accumulation in the brain is largely responsible for the problem of Alzheimer's disease (Martyn *et al.*, 1989). This is a chronic condition that is characterized by progressive loss of memory and other brain functions of daily living. It is the most common type of dementia and most cases occur after the age of 65 years. Aluminium may also be linked to multiple sclerosis and Parkinson´s disease (Piccardo *et al.*, 1998). Due to the fore said reasons, it is time an alternative safe, inexpensive and available water clarifier is investigated. In Sudan, local plants including *Maerua subcordata, Moringa stenopetala* and

Moringa oleifera (Moringaceae) are widely used in water purification (Jahn, 1979; 1981; 1986; 1991). Moringa plant seeds contain components that have proved efficient in water purification (Gassenschmidt *et al.*, 1995), as a substitute to aluminium sulphate and other flocculents. There is a dual advantage to this property: (i) it can be used as a locally produced substitute for imported flocculant, thus reducing expenditure by poor: (ii) Moringa plant flocculent, unlike aluminium sulphate, is completely biodegradable. Some farmers in Kisumu District are growing *Moringa spp* having been introduced some twenty years ago (Muluvi, 1998)

1.4 Research objectives

1.4.1 General objective

To establish antimicrobial activity and water purification ability of *M. oleifera* and *M. stenopetala.*

1.4.2 Specific objectives

1. To establish the antibacterial activity of seed extracts of *M. oleifera* and *M. stenopetala* on microorganisms (*Salmonella typhii, Vibrio cholerae* and *Escherichia coli*)
2. To compare the effectiveness of seed extracts of *M. oleifera* and *M. stenopetala* with aluminium sulphate in turbid water clarification.

1.4.3 Hypotheses

1. *M. oleifera* and *M. stenopetala* seed extracts have antibacterial effects on *Salmonella typhii, Vibrio cholerae* and *Escherichia coli*
2. *M. oleifera* and *M. stenopetala* seed extracts are effective in turbid water clarification.

CHAPTER TWO

LITERATURE REVIEW

2.1 Sources of drinking water

Sources of drinking water include deep groundwater, shallow ground water, upland lakes and reservoirs and surface water. The water emerging from some deep ground waters may have fallen as rain many decades or even hundreds of years ago. Soil and rock layers naturally filter the groundwater to a high degree of clarity before it is pumped to the treatment plant. Such water may emerge as springs, artesian springs, or may be extracted from boreholes or wells (Rick, 1998). Deep groundwater is generally of very high bacteriological quality (i.e., a low concentration of pathogenic bacteria such as *Campylobacter* or the pathogenic protozoa *Cryptosporidium* and *Giardia*) but may be rich in dissolved solids, especially carbonates and sulphates of calcium and magnesium. Shallow ground water, emerging from shallow ground is usually abstracted from wells or boreholes. The bacteriological quality can be variable depending on the nature of the catchments. A variety of soluble materials may be present including potentially toxic metals such as zinc, lead and copper (Cheserek, 2005; Mataka, *et al.*, 2006). Upland lakes and reservoirs, typically located in the headwaters of river systems, are usually sited above any human habitation and may be surrounded by a protective zone to restrict the opportunities for contamination. The levels of bacteria and other pathogens are usually low. Where uplands are forested or peaty, humic acids can colour them, thus any upland sources have low p*H* which require adjustment. Surface water used for drinking is typically obtained from ponds, rivers, springs, wells and the lake. It is regularly contaminated with both human and animal feaces (Makutsa *et al.*, 2001).

2.2 Conventional Water purification.

Water purification is the removal of contaminants from raw water to produce drinking water that is pure enough for human consumption or for industrial use. Substances that are removed during the process include parasites (such as *Giardia* or *Cryptosporidium*), bacteria, algae, viruses, fungi, minerals (including toxic metals such as lead, copper etc.), and man-made chemical pollutants (Rangel *et al.*, 2003; Souter *et al.*, 2003; Mataka, *et al.*, 2006). Many contaminants can be dangerous but depending on the quality standards, others are removed to improve the water's smell, taste, and appearance. A small amount of disinfectant is usually left in the water at the end of the purification process to reduce the risk of re-contamination in the distribution system. Many environmental and cost considerations affect the location and design of water purification plants (Schultz and Okun, 1983).

Groundwater is cheaper to treat, but aquifers usually have limited output and can take thousands of years to recharge. Surface water sources should be carefully monitored for the presence of unusual types or levels of microbial/disease causing contaminants. Procedures such as boiling or the use of a household charcoal filter are not sufficient to clarify water from an unknown source. Even natural spring water is no longer considered safe for all practical purposes (Tumwine, 2005). The common practice for clarifying surface water is by settling and decanting, cloth filtration, and coagulation with aluminium to achieve modest reductions in turbidity (Makutsa *et al.*, 2001).

Occasionally, disinfection by boiling or chemical measures by use of 1% sodium hypochlorite solution (marketed as Klorin® or WaterGuard® in Kenya) is also used. Sodium hypochlorite has always been used though it is less effective in highly turbid water and does not remove turbidity, but

adversely affects taste. A more recent water purification intervention technology in the area (Winam Division) is the flocculant-disinfectant (Crump *et al.*, 2005). The flocculant-disinfectant technology is formulated in single use sachets to work quickly on small volumes of water. One packet contains enough calcium hypochlorite to leave a residual chlorine concentration of 3.5 mg/l in 10 l of de-mineralized water. The water aggregates and facilitates the removal of suspended organic matter, bacteria, viruses, parasites, and heavy metals in treated water (Rangel *et al.*, 2003; Souter *et al.*, 2003; Mataka, *et al.*, 2006).

The first step in purification of surface water is to remove large debris such as sticks, leaves, trash and other large particles which may interfere with subsequent purification steps. This is followed by pre-conditioning, where water rich in hardness salts are treated with soda-ash (Sodium carbonate) to precipitate calcium carbonate out utilizing the common ion effect (Souter *et al.*, 2003; Neon, *et al.*, 2005). There are a wide range of techniques that can be used to remove the fine solids, micro-organisms and some dissolved inorganic and organic materials (Rangel *et al.*, 2003). The choice of method will depend on the quality of the water being treated, the cost of the treatment process and the quality standards expected (Niewohner, *et al.*, 1997). If the water is acidic, lime or soda ash is added to raise the p*H*. Lime is the more common of the two additives because it is cheaper, but it also adds to the resulting water hardness. Making the water slightly alkaline ensures that coagulation and flocculation processes work effectively and also helps to minimize the risk of lead being dissolved from lead pipes and lead solder in pipe fittings (Mataka, *et al.*, 2006).

Coagulation and flocculation are clarification methods that work by using chemicals which effectively "glue" small suspended particles together, so that they settle out of the water or stick to sand or other granules in a granular media filter. Many of the suspended particles are negatively charged. The charge keeps them suspended because they repel particles with similar charges.

Coagulation works by eliminating the natural electrical charge of the suspended particles so they attract and stick to each other. The joining of the small particles so that they will form larger particles that can settle is called flocculation (Ching, *et al.*, 1994; Crump, *et al.*, 2004). The larger formed particles are called floc. The coagulation chemicals are added in a tank (often called a rapid mix tank or flash mixer), which typically has rotating paddles. In most clarification plants, the mixture remains in the tank for 10 to 30 minutes to ensure full mixing. The amount of coagulant that is added to the water varies widely due to the different source of water and its quality. One of the more common coagulants used is aluminium sulphate, sometimes called filter alum. Aluminium sulphate reacts with water to form flocs of aluminium hydroxide. Coagulation with aluminium compounds may leave a residue of aluminium in the clarified water. This is normally about 0.1 to 0.15 mg/L (Fred *et al.*, 1992; Aziz, *et al.*, 2007; Christopher, *et al*, 2009; Vepsalaineu, *et al.*, 2009).

It is known that aluminium can be toxic to humans at high concentrations (Martyn, *et al.*, 1989). Iron (II) sulfate or iron (III) chloride, are other common coagulants. Iron (III) coagulants work over a larger p*H* range than aluminium sulphate but are not effective with water from different sources. Other benefits of iron (III) are lower costs and in some cases slightly better removal of natural organic contaminants from some waters. Coagulation with iron compounds typically leaves a residue of iron in the finished water (O´Connor, 1971). This may impart a slight taste to the water, and may cause brownish stains on porcelain fixtures. The trace levels of iron are not harmful to humans, and indeed provide a needed trace mineral. Because the taste and stains may lead to customer complaints, aluminium tends to be favoured over iron for coagulation. In flocculation, coagulants are used to destabilize the particles (Cox, *et al.*, 2005). The chosen coagulant and the raw water are slowly mixed in a large tank called a flocculation basin. Unlike a rapid mix tank, the flocculation paddles turn very slowly to minimize turbulence (Tumwine, 2005). If sedimentation is

the intended clarification method, the principle involved is to allow as many particles to collide with other particles as possible generating large and robust floc particles. Generally, the retention time of a flocculation basin is at least 30 minutes with speeds between 15 to 45 cm / minute. Flow rates less than 15 cm/min cause undesirable floc settlement within the basin.

Water exiting the flocculation basin may enter the sedimentation basin, also called a clarifier or settling basin. It is a large tank with slow flow, allowing floc to settle to the bottom. The sedimentation basin is best located close to the flocculation basin so the transit between them does not permit settlement or floc break up. Sedimentation basins can be in the shape of a rectangle, where water flows from end to end, or circular where flow is from the center outward. Sedimentation basin outflow is typically over a weir so only a thin top layer-furthest from the sediment-exits (Mark *et al.,* 2007). The amount of floc that settles out of the water is dependent on the time the water spends in the basin and the depth of the basin. The retention time of the water must therefore be balanced against the cost of a larger basin. The minimum clarifier retention time is normally 4 hours (Fred *et al.,* 1992; Aguilar, *et al.*, 2005).

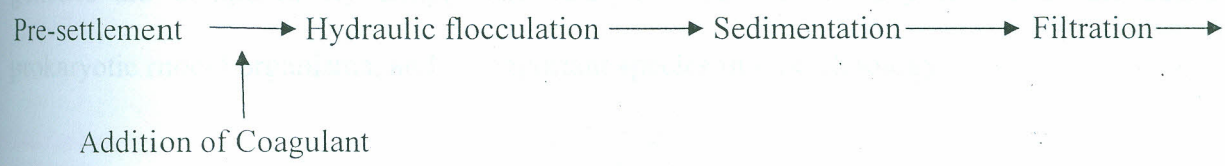
A deep basin will allow more floc to settle out than a shallow basin. This is because large particles settle faster than smaller ones, so large particles bump into and integrate smaller particles as they settle. In effect, large particles sweep vertically though the basin and clean out smaller particles on their way to the bottom. As particles settle to the bottom of the basin a layer of sludge is formed on the floor of the tank. This layer of sludge must be removed and treated. The amount of sludge that is generated is significant, often 3%-5% of the total volume of water that is treated (Mark *et al.*, 2007).

After separating most flocs, the water is filtered as the final step to remove remaining suspended particles and unsettled flocs. The most common type of filter is a rapid sand filter. Water moves

vertically through sand which often has a layer of activated carbon or anthracite coal above the sand. The top layer removes organic compounds, which contribute to taste and odour (Souter *et al.*, 2003). The space between sand particles is larger than the smallest suspended particles, so simple filtration is not enough. Most particles pass through surface layers but are trapped in pore spaces or adhere to sand particles. Effective filtration extends into the depth of the filter. This property of the filter is key to its operation: if the top layer of sand were to block all the particles, the filter would quickly clog. To clean the filter, water is passed quickly upward through the filter, opposite the normal direction (called backflushing or backwashing) to remove embedded particles. Prior to this, compressed air may be blown up through the bottom of the filter to break up the compacted filter media to aid the backwashing process; this is known as air scouring. This contaminated water can be disposed of, along with the sludge from the sedimentation basin, or it can be recycled by mixing with the raw water entering the plant (Baumann, 1978).

Some water purification plants employ pressure filters. These work on the same principle as rapid gravity filters differing in that the filter medium is enclosed in a steel vessel and the water is forced through it under pressure. Slow sand filters may be used where there is sufficient land and space. These rely on biological treatment processes for their action rather than physical filtration. Slow sand filters are carefully constructed using graded layers of sand with the coarsest at the top and finest at the base. Drains at the base convey treated water away for disinfection. Filtration depends on the development of a thin biological layer on the surface of the filter. Ultra-filtration membranes are a relatively new development; they use polymer film with chemically formed microscopic pores that can be used in place of granular media to filter water effectively without coagulants. The type of membrane media determines how much pressure is needed to drive the water through and what sizes of micro-organisms can be filtered out (Baumann, 1978; Hill and Langdon, 1991).

Disinfection is normally the last step in purifying drinking water (Illustration below). Water is disinfected to kill any pathogens which pass through the filters (Crump, *et al.*, 2004). Possible pathogens include viruses, bacteria, including *Escherichia coli*, *Campylobacter* and *Shigella*, and protozoans, including *Girdia lamblia* and other *Cryptosporidia* (Gehlbach, *et al.*, 1973). In most developed countries, public water supplies are required to maintain a residual disinfecting agent throughout the distribution system, in which water may remain for days before reaching the consumer (Rick, 1998).



Disinfection/Chlorination (Adapted from Baumann, 1978).

2.3 Water borne diseases

2.3.1 Gastroenteritis

Bacterial gastroenteritis is a very common disorder. It has several causes and ranges from mild to severe cases. It is manifested with symptoms such as vomiting, diarrhea and abdominal discomfort. Bacteria employ several mechanisms to invoke a pathological response. Virulent strains of *Escherichia coli* can cause gastroenteritis, urinary tract infections, and neonatal meningitis. In rarer cases, virulent strains are also responsible for hæmolytic-uraemic syndrome (HUS), peritonitis, mastitis, septicemia and Gram-negative pneumonia (Todar, 2007). As long as *E. coli* do not acquire genetic elements encoding for virulence factors, they remain benign commensals (Evans *et al.*, 2007).

E. coli was discovered by a German pediatrician and bacteriologist Theodor Escherich in 1885 (Feng *et al.*, 2002), and is now classified as part of the Enterobacteriaceae family of gamma-proteobacteria. *Escherichia coli* (commonly *E. coli*) are gram –negative, facultative anaerobic and non sporulating bacterium that is commonly found in the lower intestine of warm-blooded animals. *E. coli* are not always confined to the intestine, and their ability to survive for brief periods outside the body makes them an ideal indicator organism to test environmental samples for feacal contamination (Feng *et al.*, 2002; Thompson, 2007). The bacteria can also be grown easily and its genetics are comparatively simple and easily-manipulated, making it one of the best-studied prokaryotic model organisms, and an important species in biotechnology.

Most *E. coli* strains are harmless, but some, such as serotype O157:H7, can cause serious food poisoning in humans, and are occasionally responsible for costly product recalls (Vogt and Dippold, 2005; CDC , 2007). *E. coli* serotype O157:H7 is a rod shaped gram-negative bacterium, unusually virulent food-borne pathogen found primarily in cattle and causes severe sometimes life-threatening illness in man. O-refers to the somatic antigen number, H-refers to flagella antigen. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2 (Bentley and Maganathan, 1982) or by preventing the establishment of pathogenic bacteria within the intestine (Hudault *et al.*, 2001; Reid *et al.*, 2001).

A strain of *E. coli,* is a sub-group within the species, that has unique characteristics that distinguish it from other *E. coli* strains. These differences are often detectable only on the molecular level; however, they may result in changes to the physiology or lifecycle of the bacterium. For example, a strain may gain pathogenic capacity, the ability to use a unique carbon source, the ability to inhabit a particular ecological niche or the ability to resist antimicrobial agents. Different strains of *E. coli*

are often host-specific, making it possible to determine the source of fecal contamination in environmental samples (Sabin, 2006). Depending on which *E. coli* strains are present in a water sample, for example, assumptions can be made about whether the contamination originated from a human, other mammal or bird source.

New strains of *E. coli* evolve through the natural biological process of mutation, and some strains develop traits that can be harmful to a host animal. Although virulent strains typically cause no more than a bout of diarrhea in healthy adult humans, certain highly virulent strains, such as O157:H7 can cause serious illness or death in the elderly, the very young or the immunocompromised (Hudault *et al.*, 2001). *E. coli* uses mixed-acid fermentation in anaerobic conditions, producing lactate, succinate, ethanol, acetate and carbon dioxide. Since many pathways in mixed-acid fermentation produce hydrogen gas, these pathways require the levels of hydrogen to be low, as is the case when *E. coli* lives together with hydrogen-consuming organisms such as methanogens or sulfate-reducing bacteria (Madigan and Martinko, 2006).

Optimal growth of *E. coli* occurs at 37^0C, but some laboratory strains can multiply at temperatures of up to 49^0C (Fordar *et al.*, 2005). Growth can be driven by aerobic or anaerobic respiration, using a large variety of redox pairs, including the oxidation of pyruvic acid, formic acid, hydrogen and amino acids, and the reduction of substrates such as oxygen, nitrate, dimethyl sulfoxide and trimethylamine N-oxide (Ingledew and Poole, 1984). Strains that possess flagella can swim and are motile, but other strains lack flagellum. The flagella of *E. coli* have a peritrichous arrangement (Darnton *et al.*, 2007). *E. coli* and related bacteria possess the ability to transfer DNA via bacterial conjugation, transduction or transformation, which allows genetic material to spread horizontally through an existing population. This process led to the spread of the gene encoding shiga toxin from *Shigella* to *E. coli* O157:H7, carried by a bacteriophage (Brussow *et al.*, 2004).

E. coli normally colonizes an infant's gastrointestinal tract within 40 hours of birth, arriving with food or water or with the individuals handling the child. In the bowel, it adheres to the mucus membrane of the large intestine. It is the primary facultative organism of the human gastrointestinal tract (Todar, 2007). Enteric *E. coli* (EC) are classified on the basis of serological characteristics and virulence properties (Todar, 2007). Virotypes include; Enterotoxigenic *E. coli* (ETEC), the causative agent of diarrhea (without fever) in humans, pigs, sheep, goats, cattle, dogs, and horses. Enteropathogenic *E. coli* (EPEC), causes diarrhea in humans, rabbits, dogs, cats and horses. Like ETEC, EPEC also causes diarrhea, but the molecular mechanisms of colonization and etiology are different. Enteroinvasive *E. coli* (EIEC) is found only in humans. EIEC infection causes a syndrome that is identical to Shigellosis, with profuse diarrhea and high fever. Enterohaemorrhagic *E. coli* (EHEC) is found in humans, cattle, and goats. The sole member of this virotype is strain O157:H7, which causes bloody diarrhea and no fever. EHEC can cause haemolytic-uraemic syndrome and sudden kidney failure. Enteroaggregative *E. coli* (EAEC) is found only in humans.

Certain strains of *E. coli*, such as O157:H7, produce toxins. Food poisoning caused by *E. coli* is usually associated with eating unwashed vegetables and meat contaminated post-slaughter. This strain is further notorious for causing serious and even life-threatening complications like haemolytic-uraemic syndrome (HUS). Severity of the illness varies considerably, it can be fatal, particularly to young children, the elderly or the immunocompromised, but is more often mild. *E. coli* can harbor both heat-stable and heat-labile enterotoxins. The latter, termed LT, contains one 'A' subunit and five 'B' subunits arranged into one holotoxin, and is highly similar in structure and function to cholera toxins. The 'B' subunits assist in adherence and entry of the toxin into host intestinal cells, while the 'A' subunit is cleaved and prevents cells from absorbing water, causing diarrhea. LT is secreted by the Type 2 secretion pathway (Tauschek *et al.*, 2002).

If *E. coli* bacteria escape the intestinal tract through a perforation (for example from an ulcer, a ruptured appendix, or a surgical error) and enter the abdomen, they usually cause peritonitis that can be fatal without prompt treatment. However, *E. coli* are extremely sensitive to such antibiotics as streptomycin or gentamycin. This could change since *E. coli* like other pathogenic microorganisms quickly acquire drug resistance (Chandarana *et al.*, 2005). Recent research suggests that treatment with antibiotics does not improve the outcome of the disease, and may in fact significantly increase the chance of developing haemolytic uraemic syndrome (Wong *et al.*, 2000). Intestinal mucosa-associated *E. coli* are observed in increased numbers in the inflammatory bowel diseases, Crohn's disease and ulcerative colitis (Rolhin *et al.*, 2007). Invasive strains of *E. coli* exist in high numbers in the inflamed tissue, and the number of bacteria in the inflamed regions correlates to the severity of the bowel inflammation (Baumgart *et al.*, 2007).

Transmission of pathogenic *E. coli* often occurs via fecal-oral transmission (Gehlbach *et al.*, 1973; Evans *et al.*, 2007). Common routes of transmission include, unhygienic food preparation, farm contamination due to manure fertilization (Sabin, 2006), irrigation of crops with contaminated grey water or raw sewage (Heaton and Jones, 2007), feral pigs on cropland (Thompson, 2007), or direct consumption of sewage-contaminated water (Chalmers *et al.*, 2000). Dairy and beef cattle are primary reservoirs of *E. coli* O157:H7 (Bach *et al.*, 2002), and they can carry it asymptomatically and shed it in their feces (Bach *et al.*, 2002). Food products associated with *E. coli* outbreaks include raw ground beef, raw seed sprouts or spinach, raw milk, unpasteurized juice, and foods contaminated by infected food workers via fecal-oral route (Sabin, 2006).

Uropathogenic *E. coli* (UPEC) is responsible for approximately 90% of urinary tract infections (UTI) seen in individuals with ordinary anatomy (Todar, 2007). In ascending infections, fecal bacteria colonize the urethra and spread up the urinary tract to the bladder. Because women have a shorter urethra than men, they are 14-times more likely to suffer from an ascending UTI (Todar, 2007). Uropathogenic *E. coli* utilize P-fimbriae (pyelonephritis-associated pili) to bind urinary tract endothelial cells and colonize the bladder. These adhesins specifically bind D-galactose-D-galactose moieties on the P-blood group antigen of erythrocytes and uroepithelial cells (Todar, 2007). Approximately 1% of the human population lacks this receptor, and its presence or absence dictates an individual's susceptibility to *E. coli* urinary tract infections. Uropathogenic *E. coli* produce alpha- and beta-hemolysins, which cause lysis of urinary tract cells. UPEC can evade the body's innate immune defenses (e.g. the complement system) by invading superficial umbrella cells to form intracellular bacterial communities (IBCs) (Ehrlich *et al.*, 2005). They also have the ability to form K-antigen, capsular polysaccharides that contribute to biofilm formation. Biofilm-producing *E. coli* are recalcitrant to immune factors and antibiotic therapy and are often responsible for chronic urinary tract infections (Ehrlich *et al.*, 2005). K-antigen-producing *E. coli* infections are commonly found in the upper urinary tract (Todar, 2007). Descending infections, though relatively rare, occur when *E. coli* cells enter the upper urinary tract organs (kidneys, bladder or ureters) from the blood stream.

Bacterial infections are usually treated with antibiotics. However, the antibiotic sensitivities of different strains of *E. coli* vary widely. As Gram-negative organisms, *E. coli* are resistant to many antibiotics that are effective against Gram-positive organisms. Antibiotics which may be used to treat *E. coli* infection include amoxicillin as well as other semi-synthetic penicillins, many cephalosporins, carbapenems, aztreonam, trimethoprim-sulfamethoxazole, ciprofloxacin,

nitrofurantoin and the aminoglycosides. Antibiotic resistance is a growing problem. Some of this is due to overuse of antibiotics in humans, but some of it is probably due to the use of antibiotics as growth promoters in food of animals (Johnson *et al.*, 2006).

Antibiotic-resistant *E. coli* may also pass on the genes responsible for antibiotic resistance to other species of bacteria, such as *Staphylococcus aureus*. *E. coli* often carry multidrug resistant plasmids and when under stress, readily transfer those plasmids to other species. Indeed, *E. coli* is a frequent member of biofilms, where many species of bacteria exist in close proximity to each other. This mixing of species allows *E. coli* strains that are piliated to accept and transfer plasmids from and to other bacteria. Thus *E. coli* and the other enterobacteria are important reservoirs of transferable antibiotic resistance (Salyers *et al.*, 2004). Resistance to beta-lactam antibiotics has become a particular problem in recent decades, as strains of bacteria that produce extended-spectrum beta-lactamases have become more common (Paterson and Bonomo, 2005). These beta-lactamase enzymes make many, if not all, of the penicillins and cephalosporins ineffective as therapy. Extended-spectrum beta-lactamase-producing *E. coli* are highly resistant to an array of antibiotics and infections by these strains are difficult to treat. In many instances, only two oral antibiotics and a very limited group of intravenous antibiotics remain effective. Researchers have actively been working to develop safe, effective vaccines to lower the worldwide incidence of *E. coli* infection (Girard *et al.*, 2006). In March 2006, a vaccine eliciting an immune response against the *E. coli* O157:H7 O-specific polysaccharide conjugated to recombinant exotoxin A of *Pseudomonas aeruginosa* (O157-rEPA) was reported to be safe in children; two to five years old. Previous work had already indicated that it was safe for adults (Ahmed *et al.*, 2006). So the efficacy of *M. oleifera* and *M. stenopetala* preparations should be tested against *E. coli*.

2.3.2 Typhoid

Salmonella enterica (*S. enterica*) is a Gram-negative facultative rod-shaped bacterium in the same proteobacterial family as *Escherichia coli,* the family *Enterobacteriaceae,* trivially known as "enteric" bacteria. *Salmonella* is nearly as well studied as *E. coli* from a structural, biochemical and molecular point of view, and as poorly understood as *E. coli* from an ecological point of view. Salmonellae live in the intestinal tracts of warm and cold-blooded animals. Some species are ubiquitous. Other species are specifically adapted to a particular host. *S. enterica* has an extraordinarily large number of *serovars* or strains, up to 2000 have been described (Ryan and Ray, 2004). *Salmonella enterica* Serovar Typhi (historically elevated to species status as *S. typhii*) is the disease agent in typhoid fever, resulting from bacterial invasion of the blood stream of man through the intestinal lining, mostly via ulcerations. Other serovars such as Typhimurium (also known as *S. typhimurium*) can lead to a form of human gastroenteritis sometimes referred to as salmonellosis, resulting from a food borne infection/intoxication in humans.The genome sequences of serovars *typhii* (Parkhill, 2001) and *typhimurium* LT2 (McClelland, 2001) have been established. *Salmonella typhii* is a serovar of *Salmonella enterica* (formerly known as *Salmonella choleraesuis*) and the cause of the disease typhoid fever. The organism can be transmitted by the fecal oral route it is excreted by humans in feces and may be transmitted by contaminated water, food, or by person to person contact (with inadequate attention to personal hygiene).

Most cases of salmonellosis are caused by food infected with *S. enterica*, which often infects cattle and poultry, though also other animals such as domestic cats and hamsters (Swanson *et al.*, 2007) have also been shown to be sources of infection to humans. However, investigations of vacuum cleaner bags have shown that households can act as a reservoir of the bacterium. This is more likely if the household has contact with an infection source, for example through members working with

cattle or in a veterinary clinic. Raw chicken and goose eggs can harbor *Salmonella enterica*, initially in the whites of the eggs, although most eggs are not infected. As the egg ages at room temperature, the yolk membrane begins to break down and *Salmonella enterica* can spread into the yolk. Refrigeration and freezing do not kill all the bacteria, but substantially slow or halt their growth. Pasteurizing (briefly heating to a specific temperature) and irradiation are used to kill salmonella for commercially produced foodstuffs containing raw eggs such as ice cream. Foods prepared in the home from raw eggs such as mayonnaises, cakes and cookies can spread salmonella if not properly cooked before consumption.

2.3.3 Cholera

Cholera, sometimes known as Asiatic cholera or epidemic cholera, is an infectious gastroenteritis caused by the bacterium *Vibrio cholerae* (Ryan and Ray, 2004; Faruque and Nair, 2008). Transmission to humans occurs through ingesting contaminated water or food. The major reservoir for cholera was long assumed to be humans themselves, but considerable evidence exist that aquatic environment can serve as reservoirs of the bacteria. *Vibrio cholerae* is a Gram-negative bacterium that produces cholera toxin, an enterotoxin, whose action on the mucosal epithelium lining of the small intestine is responsible for the characteristic massive diarrhea of the disease (Ryan and Ray, 2004). In its most severe forms, cholera is one of the most rapidly fatal illnesses known, and a healthy person may become hypotensive within an hour of the onset of symptoms. Infected patients may die within three hours if treatment is not provided (Ryan and Ray, 2004). In a common scenario, the disease progresses from the first liquid stool to shock in 4 to 12 hours, with death following in 18 hours to several days without oral rehydration therapy (McLeod, 2000; WHO, 2007). The diarrhea associated with cholera is acute and so severe that, unless oral rehydration therapy is started promptly, the diarrhea may within hours result in severe dehydration or even

death. Other symptoms include rapid pulse, dry skin, tiredness, abdominal cramps, nausea, and vomiting.

Water and electrolyte replacement in the body of the patient is an essential treatment against cholera, as dehydration and electrolyte depletion occur rapidly due to vomiting and diarrhea. The use of intravenous rehydration may be absolutely necessary in severe cases, under some conditions. In addition, tetracycline is typically used as the primary antibiotic, although some strains of *Vibrio cholerae* exist that have shown resistance. Other antibiotics that have been proven effective against *Vibrio cholerae* include cotrimazole, erythromycin, doxycycline, chloramphenicol, and furazole. Fluoroquinolones such as norfloxacin also may be used, but resistance has been reported (Hemendra *et al.*, 2006). Rapid diagnostic assay methods are available for the identification of multidrug resistant *Vibrio cholerae* (Mackay, 2007). New generation antimicrobials have been discovered which are effective against *Vibrio cholerae* in *in vitro* studies (Ramamurthy, 2008).

Some of the preventive measures to the spread of *Vibrio cholerae* include, sterilization of all materials (such as clothing and bedding) that come in contact with cholera patients in hot water using chlorine bleach if possible. Hands that touch cholera patients or their clothing and bedding should be thoroughly cleaned and sterilized. Treatment of general sewage before it enters the waterways or underground water supplies. All water used for drinking, washing, or cooking should be sterilized by boiling or chlorination in any area where cholera may be present. Boiling, filtering, and chlorination of water kill the bacteria produced by cholera patients and prevent infections from spreading. Public health education and appropriate sanitation practices can also help prevent transmission.

Recent epidemiologic research suggests that an individual's susceptibility to cholera (and other diarrhoeal infections) is affected by their blood type: Those with type O blood are the most susceptible (Swerdlow *et al.*, 1994; Haris *et al.*, 2005), while those with type AB are the most resistant. Between these two extremes are the A and B blood types, with type A being more resistant than type B (Waltz and Robert, 2007). About one million *Vibrio cholerae* bacteria must typically be ingested to cause cholera in normally healthy adults, although increased susceptibility may be observed in those with a weakened immune system, individuals with decreased gastric acidity (as from the use of antacids), or those who are malnourished (Bertranpetit and Calafell, 1996).

Persons infected with cholera have massive diarrhea. This highly-liquid diarrhea is loaded with bacteria that can spread to infect water used by other people. Cholera is transmitted from person to person through ingestion of water contaminated with the cholera bacterium, usually from faeces or other effluent. The source of the contamination is typically other cholera patients when their untreated diarrhea discharge is allowed to get into waterways or into groundwater or drinking water supply. Any infected water and any foods washed in the water, as well as shellfish living in the affected waterway, can cause an infection. Cholera is rarely spread directly from person to person. *Vibrio cholerae* harbors naturally in the plankton of fresh, brackish, and salt water, attached primarily to copepods in the zooplankton. Both toxic and non-toxic strains exist. Non-toxic strains can acquire toxicity through a lysogenic bacteriophage (Huq and Xu, 1996).

Most of the *Vibrio cholerae* bacteria in the contaminated water that a host drinks do not survive the very acidic conditions of the human stomach (Hartwell *et al.*, 2004). The few bacteria that do survive conserve their energy and stored nutrients during the passage through the stomach by shutting down much protein production. When the surviving bacteria exit the stomach and reach the

small intestine, they need to propel themselves through the thick mucus that lines the small intestine to get to the intestinal wall where they can thrive. On reaching the intestinal wall, *Vibrio cholerae* start producing the toxic proteins that give the infected person watery diarrhea. This carries the multiplying new generations of *Vibrio cholerae* bacteria out into the drinking water of the next host if proper sanitation measures are not in place.

Cholera was originally endemic to the Indian subcontinent, with the Ganges River likely serving as a contamination reservoir. The disease spread by trade routes (land and sea) to Russia, then to Western Europe, and from Europe to North America. Cholera is now no longer considered a pressing health threat in Europe and North America due to filtering and chlorination of water supplies, but affects heavily populations in developing countries, Kenya included. In Nyanza, Kisumu District and Winam Division, cholera is a health problem. Data made available from Kisumu District Hospital Microbiology Laboratory Records indicated that between 14/06/08 to 29/07/08, there were 214 suspected cases of cholera reported. Out of this, 121 (57%) cases proved positive (had growth) while 93 (43%) cases had no growth. The growths obtained were *Vibrio cholerae* strains, *ogawa, inaba* and *polyvalent.* The most affected areas included, Manyatta, Obunga, Kajulu, Kasagam, Kondele, Bandani, Nyalenda, Kolwa, Kibos prison, Rabuor, Miwani and Ahero. The efficacy of *M. oleifera* and *M. stenopetala* preparations against *Vibrio cholerae* should therefore be tested since it is cheaper alternative to antibiotics and can be made readily available to the local population.

2.4 Water purifying plants

Natural materials have been used in water purification since ancient times. But lack of knowledge on the exact nature and mechanism by which they work has impeded their widespread application. In recent years there has been increased interest to use natural materials in water purification due to cost and associated health and environmental concerns of synthetic organic and inorganic chemicals. A number of effective coagulants have been identified from plant materials. Such plants include; *Cactus latifaira* and *Prosopis juliflora* (Diaz, *et al.*, 1999), tannin from Valonia (Ozacar and Sengil, 2000), *Maerua subcordata* (Gottsch, 1984; Beentje, 1994; Swaleh, 1999; Aschalew and Adinew, 2004), *Moringa oleifera* and *M. stenopetala* (Noad and Birnie, 1989; Gassenchmidt *et al.*, 1995; Maundu *et al.*, 1999).

2.4.1 *Moringa oleifera*

The *Moringa oleifera* is also known as horseradish tree (Morton, 1991), drumstick tree, Ben-oil tree and mother's best friend. It is a small, fast-growing, drought tolerant deciduous tree that ranges in height from 5-12 m with an open, umbrella shaped crown, straight trunk (10-30cm thick) with corky, whitish bark (Schwarz, 2000). The evergreen foliage (depending on climate) has leaflets 1-2 cm in diameter; the flowers are fragrant, white or cream colored 2.5cm in diameter, stamens yellow. The fruits (pods) are pendulous, initially light green, slim and tender, eventually becoming dark green, firm and up to 120 cm long, 1.8cm wide, depending on the variety (Plate 1).

Plate. 1. Fresh *M. oleifera* leaves, flowers and mature pod as collected from a farmer in Kajulu Location (Photograph by Author).

Fully mature pods carry about 20 dried seeds which are round or triangular shaped. The seeds are dark brown, the kernel being surrounded by a lightly wooded shell with three papery wings. It tends to be deeply rooted, has a wide-open typically-umbrella shaped crown and usually a single stem (Dishna, 2000; Katende, *et al*, 2000; Schwarz, 2000; Maundu and Tengna□s, 2005). *Moringa oleifera* belongs to the family of shrubs and trees, the moringaceae. The seeds are used in water purification (Sutherland, *et al*., 1990; Sutherland, *et al*., 1994), leaves used as vegetable (Ramachandran *et al*., 1980) and fodder, it has antimicrobial substances (Eilert *et al*., 1981) and the seed produces edible oil (Khan, *et al*., 1995).

Moringa spp tolerate annual precipitation of 250 to 1500 mm, annual temperature of 26-40°C and a soil p*H* of 4.5 to 8 (Duke, 1978). *Moringa spp* is propagated by planting stem/branch cuttings 1 to 2m long, root and seeds. The plants start bearing seed pods 6 to 8 months after planting. Although a native species of northern India, the tree is now grown extensively throughout the tropics and is found in many countries of Africa, Asia and South America. Planting of *M. oleifera* in East Africa has increased over the last four years and seeds for planting in Kenya, Tanzania and lately Uganda, are obtained from Mbololo, Kenya. In Kenya *Moringa oleifera* is abundantly found in Baringo district, Makindu and Malindi at the coast, in Kisumu District and Bondo District (in Usenge), Nyanza Province (Dishna, 2000; Katende, *et al*, 2000; Maundu and Tengnás, 2005).

2.4.2 *Moringa stenopetala*

M. stenopetala is also a tree measuring between 6-10 m tall; trunk: more or less 60 cm in diameter at breast height; crown: strongly branched sometimes with several branches; thick at base; bark: white to pale gray or silvery, smooth; wood: soft; leaves: up to 55 cm long; inflorescence: pubescent, dense many flowered panicles calyx 60 cm long (Jahn, 1991; Schwarz, 2000; Maundu *et al.*, 2005). *M. stenopetala* seeds have better water purifying properties than *M. oleifera.*

Apart from water purifying qualities (Mayer and Stelz, 1992), *Moringa* pods are an important commercial vegetable crop throughout India (Ramachandran, *et al.*, 1980; Olsen, 1987). The leaves have a high protein content of 27% and are rich in vitamins A and C, calcium, iron and phosphorus (Fuglie, 1999). A particular advantage is that *Moringa stenopetala* leaves can be harvested during the dry season when no other fresh vegetables are available (Duke, 1987; Morton, 1991). Its seeds contain 40% of oil by weight. *Moringa spp* seeds can be used first for oil extraction, without reducing their effectiveness for water treatment. *Moringa spp* oil is of high quality and potentially

has a high market value. The oil is of equal value both for cooking oil and as the main ingredient for soap manufacture (Folkard and Sutherland, 2005). It is found in Ethiopia and Kenya. In Ethiopia it is only found under cultivation while in Kenya *M. stenopetala* is reported to grow as a wild tree around Lake Baringo, Isiolo, Marsabit and Turkana Districts (Folkard and Sutherland, 2005; Maundu *et al.*, 2005).

2.5 Water Borne Disease Treatment and Prevention using Moringa Plant.

The benefits for the treatment or prevention of disease or infection that may accrue from either dietary or topical administration of Moringa preparations (e.g. extracts, powders, porridges) are not quite so well known (Tesemma, *et al.*, 1993). Although the oral history here is also voluminous, it has been subject to much less intense scientific scrutiny, and it is useful to review the claims that have been made and to assess the quality of evidence available for the better documented claims. Widespread claims of the medicinal effectiveness of various Moringa tree preparations have encouraged researcher to further investigate some of these possibilities.

A plethora of traditional medicine references attest to its curative power, and scientific validation of these popular uses is developing to support at least some of the claims. Moringa tree preparations have been cited in the scientific literature as having antibiotic (Eilert *et al.*, 1981; Nwosu and Okafor, 1995; Mashiar *et al.*, 2009), antitrypanosomal (Mekonnen *et al.*, 1999), antihelminths (Fuglie, 1999; 2001), hypotensive (Jadhav, 2000), antispasmodic (Faizi, 1998), anti-ulcer (Caceres and Lopez., 1991), anti-inflammatory (Jadhav, 2000), hypocholesterolemic (Ghasi *et al.*, 2000) and hypoglycemic activities (Kar and Chounddhary, 1999), HIV-AIDs management (Prazuk, 1993), anti-Herpes simplex virus type 1 (Lipipun *et al.*, 2003), anti-inflammatory (Rao, *et al.*, 1999), as well as having considerable efficacy in water purification by flocculation and sedimentation

(Gottsch, 1984; Madsen *et al.*, 1987; Gottsch, 1992), antiosis and even reduction of *Schistosome cercarie* titer (Olsen, 1987). Unfortunately, many of these reports of efficacy in human beings are not supported by placebo controlled, randomized clinical trials, nor have they been published in high visibly journals. For example, on the surface a report published almost 25 years ago appears to establish Moringa tree as a powerful cure for urinary tract infection (Shaw and Jana, 1982), but it provides the reader with no source of comparison (no control subjects).

In many cases, published in-vitro (cultured cells) and in-vivo (animals) trials do provide a degree of mechanistic support for some of the claims that have sprung from the traditional medicine lore. For example, numerous studies now point to the elevation of a variety of detoxication and antioxidants enzymes and biomarkers as a result of treatment with Moringa plant or with phytochemicals isolated from Moringa plant (Kumar and Pari, 2003). *M. oleifera* seed suspension has been used to eliminate bacteria from water for drinking (Joachim, 2004). Antibiosis and cancer prevention are the two examples of areas of Moringa research for which the existing scientific evidence appears to be particularly strong (Fuglie, 2000).

M. oleifera has demonstrated higher antibacterial activity of ethanolic extracts while aqueous extracts showed least activity on *E. coli* and *S. typhii* (Doughari, *et al.*, 2007). However efficacy of the same extracts against *V. cholerae* was not tested. It has also been demonstrated that Moringa fresh leaf juice and aqueous extracts from seeds inhibits the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Nepolean, *et al.*, 2009). A study demonstrated that crude, supernatant, residue and dialyzed seed extracts of *M. oleifera* had inhibition against *Pasturella multocida, E. coli, Bacillus subtilis* and *S. aureas* (Raheela, *et al.*, 2008). Inhibition has also been observed in *E. coli, Staphylococcus aureus, P. aeruginosa* and *Bacillus subtilis* using *Moringa oleifera* Lam steam

distillate of dried leaf powder (Kakuda, *et al.*, 2010). Antibacterial activity of n-hexane and methanol extracts of *M. oleifera* and *M. stenopetala* seeds against *E. coli*, *S. typhii* and *V. cholera* has not been tested.

2.6 Water Coagulation with Moringa seeds powder

Generally, coagulants are used for (physical and chemical) clarification of turbid raw waters. At very high turbidity, using filters can no longer adequately clarify the water. Coagulants have to be applied to transform water constituents into forms that can be separated out physically. In large-scale treatment plants aluminum sulfate is used as a conventional chemical coagulant (Francis and Amos, 2009). Natural coagulants have been used for centuries in traditional water clarification practices throughout certain areas of the developing world (Madsen *et al.*, 1987). As an alternative to conventional coagulants, *Moringa oleifera* seeds can be used as a natural coagulant (primary coagulant) in household water clarification as well as in the community water treatment systems (Olsen, 1987; Sutherland *et al.*, 1994; Francis and Amos, 2009). Natural coagulant properties were found in six different *Moringa spp* by laboratory studies.

The seed kernels of *Moringa oleifera* contain significant quantities of low molecular-weight (water-soluble components) that carry a positive charge (Gassenschmidt *et al.*, 1995). When the crushed seeds are added to raw water, the extract produce positive charges acting like magnets and attracting the predominantly negatively charged particles (such as clay, silk, and other toxic particles in water) (Sutherland *et al.*, 1990; Sutherland *et al.*, 1994). The flocculation process occurs when the extract molecules bind the negative charges forming flocs through the aggregation of particles, which are present in water. Since bacteria in water are generally attached to solid particles, clarification with *Moringa* powder can leave water clear with 90-99% of the bacteria removed (Madsen *et al.*, 1987;

Nepolean, *et al.*, 2009). Additional purification of the water by boiling or adding chlorine or bleach is needed to render it completely safe to drink. Studies have been carried out to determine the potential risks associated with the use of *Moringa* seeds in water clarification (Bergeret *et al.*, 1984). To date, no evidence has been found that the seeds cause secondary effects in humans, especially at the low doses required for water clarification (Sutherland *et al.*, 1990).

According to Jahn (1981), the seeds of Moringa species are efficient water coagulants and toxic side effects have not been noted. The active substances effective in flocculation have been gathered by chromatography to cationic protein polymers, which bring about the same effectiveness as the best technical water-clarifying agents such as alum used in waterworks (Barth *et al.*, 1982; Francis and Amos, 2009). The flocculation mechanism is initiated by electronic process whereby bridging between negative colloid particles and cationic polymers take place and the process accelerated through continuous stirring, resulting in a three dimensional aggregation. Trials by (Jahn, 1981) indicated that a concentration of 200 mg of powdered seeds/liter reduced turbidity (total solids 8,000mg/litre) after one hour by 98%. To clarify 40 liters of water, about 30 crushed seeds are needed. One mature tree can produce about 5,500 seeds per year sufficient for about 7,000 liters of water.

Considerable research work has been done on the agronomy of the Moringa trees (Sharma and Raina, 1980; Muluvi, 1998; Maundu *et al.*, 1999; Muluvi, *et al.*, 1999; Maundu and Tengnás, 2005) and more research needs to be done on the best means to produce and apply powdered seeds as water purifiers and as a source of high quality cooking oil. Preliminary results on the use of crushed seeds as water purifiers are promising (Aschalew and Adinew, 2004). It has also been noted that since many diseases are water-borne, research on these plants that are both water clarifier and at the

same time antibacterial, is very profitable (Melaku and Yohannes, 1988). Even if the water is not totally free from germs after clarification with Moringa plant seeds, the number of germs is considerably reduced (Madsen *et al.*, 1987) and the water is at least free of particles. This indeed is an important improvement of the water quality, with the help of local tree product, which at the same time produce valuable vegetables (Duke, 1987) and cooking oil (Folkard and Sutherland, 2005). In view of the fact that most pathogenic organisms are becoming resistant to antibiotics (Chandarana *et al.*, 2005), and people are dependent on turbid surface water from ponds, rivers, wells and lake to a great extent (Makutsa *et al.*, 2001), wide application of greatly under utilized trees like *M. oleifera* and *M. stenopetala* would be most desirable to use as water clarifier and disinfectant.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area.

The study area was Winam Division, within Kisumu East District which lies within longitudes 33° 20' E and 35° 20' E and latitudes 0° 20' S and 0° 50' S and covers a total area of 2660 sq. km of which 567 sq. km are under water. The District is divided into four administrative Divisions, twenty nine locations and one hundred and seventeen sublocations. The divisions are Winam, Maseno, Kadibo and Kombewa (Robinson, *et al.*, 2005). Some of the rivers flowing into Winam Division are Kibos, Auji, Kisat, and the Nyamasaria River. Winam division had a population of 350,365 by 2002 (Robinson, *et al.*, 2005). The rapid population growth after independence in 1963 has caused a successive deterioration of resident's access to clean water and sewage disposal (Jan, *et al.*, 2002), leading to prevalence of water borne diseases such as typhoid and cholera (Ouma, *et al.*, 2005; Robinson, *et al.*, 2005). Kisumu District has only 26 health facilities (Lemeshow and Hosmer, 1982). Most of the health facilities are concentrated in Kisumu town. The average distance to a health centre is 5-8 kilometres making it difficult for most people to access health facilities in the rural areas (Robinson, *et al.*, 2005). The fore said reasons made it necessary for this research to target this area for the study.

3.2 Moringa Seed collection and pre-extraction preparations

Seeds of *Moringa oleifera* were collected from a single tree grown by a farmer in Kajulu location, Winam Division, Kisumu East District. Seeds of *M. stenopetala* were obtained from Kenya Forestry Research Institute (Maseno Regional Centre). Botanical confirmation of the identified species was done at the National Museum of Kenya, Nairobi. The seeds were deshelled to remove the kernels. Seed kernels were further dried at ambient temperatures for a period of five days before milling.

The white kernels were milled into a fine powder using a Christy Laboratory Mill-8000rpm. The fine powder obtained was then sieved through a number 26 sieve.

3.3 Extraction of non-polar and polar components.

This was a two stage process:

(a) Extraction of oil using n-hexane

(b) Extraction of water soluble compound using Methanol

3.3.1 Extraction of oil using n-hexane

1000gms each of powder of *Moringa oleifera* and *Moringa stenopetala* was soaked in 2000ml n-hexane in a 5 litre Ehlenmeyer flask, corked and placed in a laboratory shaker (UAR Orbital shaker SO1) for 4 hrs and then allowed to stay overnight in order to settle down for decantation into a one litre flask. The extract was then vacuum filtered through Whatmans filter paper No.1 using water pump into a Butchner flask. The filtrate was then concentrated using a rotary evaporator (type KRvrTD 65/45) at 40°C water bath until the condensation of the solvent stopped dropping. The n-hexane extract was then transferred into a sterilized beaker.

3.3.2 Extraction using Methanol

The solid residue from section 3.3.1 added into two 5litre conical flask containing 2000ml of methanol was placed in a laboratory shaker (UAR Orbital shaker SO1) for 4 hrs and then allowed to stay overnight in order to settle down for decantation into a one litre flask. The extract was then vacuum filtered through Whatmans filter paper No.1 using water pump into a Butchner flask.. The filtrate was then concentrated using a rotary evaporator (type KRvrTD 65/45) at 40°C water bath until the condensation of the solvent stopped dropping.

3.4 Antibacterial Studies

3.4.1 Media Preparation:

The following media were prepared according to the manufacturers (HI Media Laboratory PVT Ltd) instructions.

1. Peptone bacteriological agar

10g of the powder was suspended in 1 litre of distilled water in a conical flask. It was boiled to completely dissolve in water. It was then sterilized by autoclave at 121° C for 15 minutes and transferred into sterilized disposable and pyrex petridishes, 55mm and 90mm diameters respectively. The media were allowed to cool under aseptic condition before being used.

2. McConkey agar

52g of the powder was suspended in 1 litre of distilled water in a conical flask. It was boiled to completely dissolve in water. It was then sterilized by autoclave at 121° C for 15 minutes and transferred into sterilized disposable and pyrex petridishes, 55mm and 90mm diameters respectively. The media were allowed to cool under aseptic condition before being used.

The above media were used since they are selective media for *Vibrio cholerae* and *E. coli*, *Salmonella typhii* respectively.

3.4.2 Test organisms:

The standard reference bacteria were obtained from Kenya Medical Research Institute (KEMRI), Kisian-Kisumu Centre. Three bacteria species, *Salmonella typhi*, *Escherichia coli* and *Vibrio cholerae* (ref. Romel Cary Blair Lot. 452610) were obtained.

3.4.3 Preliminary screening for Antibacterial Activity:

This was carried out using the disc diffusion method of Barry and Brown, (1996). Each extract was reconstituted in the DMSO (dimethyl sulphoxide) at dilutions 2.5, 5, 10, 20 and 40% for n-hexane extract and 2.5, 5, 10, 20 and 40% for methanol extract, to assess the antibacterial activity. A sterile cotton swab (on a wooden applicator stick) was dipped into the bacterial suspension. A swab was then evenly streaked in three directions over the entire surface of the agar plate to obtain uniform inoculums. *E. coli* and *Salmonella typhii* were inoculated onto McConkey agar while *V. cholerae* was inoculated onto Bacteriological peptone agar. Immediately after inoculation of the media, sterilized paper discs (made from Whatman 150mm, filter paper) of 5mm diameter were impregnated with the test extracts (at concentrations 2.5, 5, 10, 20, and 40% for n-hexane and 2.5, 5, 10, 20 and 40% for methanol extract) and placed onto the surface of the inoculated media. Plates set with DMSO served as controls. The experiment was replicated four times. The experiment was allowed to stand for 48 hours in an incubator at 29°C. The presence of zones of inhibition around the discs was interpreted as preliminary indication of antibacterial activity. The zones of inhibition were then measured using veneer calipers in millimeters and recorded.

3.5 Water sampling sites

3.5.1 Sampling at Dunga inlet.

Dunga water inlet is located, 0° 07′ 33.05″ S Latitude, 34° 44′ 29.43″ E Longitude and the Altitude is 3741ft (Plate 2).

Plate. 2. KIWASCO Dunga water inlet (Photograph by Author).

3.5.2 Sampling at River Kibos.

The sampling site is located, ° 04′ 14.81″ S Latitude, 34° 48′ 53.38″ E Longitude and the Altitude is 3836ft.

3.5.3 Sampling at Nyamasaria dam.

The sampling site is located, ° 07′ 23.20″ S Latitude, 34° 47′ 36.44″ E Longitude and the Altitude is 3757ft.

3.5.4 Collection of Water samples

Water samples were collected from the three different locations: Lake Victoria (Dunga KIWASCO water inlet), River Kibos and Nyamasaria Pond. Samples were collected in 5 litre sterile plastic vessels. The collection of the water samples was done at 4.00 p.m, during the rainy season when water bodies are disturbed by strong winds, animals and man, and the surface runoffs carrying

suspended particles and microbes are emptied into the water bodies increasing the turbidity of the waters.

3.6 Water clarification studies

3.6.1 Water sample Treatments

The experiment was carried out at Kisumu Water and Sewarage Company (KIWASCO) Laboratory. There were three water samples collected from Lake Victoria Dunga site, River Kibos and Nyamasaria dam. From each site, 5 litres of water was collected. This was taken to the laboratory for analysis. Using measuring cylinder, 250 ml of each water sample was measured and placed in a 500 ml sterile beaker. The p*H* of each water sample was recorded using the laboratory p*H* meter. Before the treatments, the turbidity of each water sample was taken using a turbidity meter (Hach 2100P Portable Turbidimeter). The *Moringa spp* extract and aluminum sulphate treatments (Table 1) were then placed into the beakers. The beakers were placed on a stirrer and stirred for 15 minutes. Samples were then left undisturbed for 20 minutes on the table. The clear water above the flocs was decanted into turbidity measuring bottles and the pH and turbidity recorded. The treatments were replicated three times.

3.6.2 Treatments

The treatments of the above flocculents consisted of the following quantities (Table 1 below).

Table 1 .Treatment in grams (per 250ml of water).

0.00g (Control)
0.0625 g
0.125 g
0.25 g
0.5 g
1.0 g

CHAPTER FOUR

RESULTS

4.1 Extraction of non-polar and polar components.

The extract obtained from n-hexane, was oil while the extract from methanol was semi solid.

From 1000gms of *M. oleifera* powder, a pale yellow oil of the volume 340mls and mass 301gms was obtained. The density was found to be 0.885gm/ml. A methanol extract 126mls, 84gms and density 0.7gm/ml was also recovered from *M. oleifera* residue from n-hexane extraction. The residue from methanol extraction (cake) weighed 615gms.

From 1000gms *M. stenopetala* powder, a yield of 226mls and 181gms of a pale yellow oil of density 0.8gm/ml was obtained. The methanol extract of 67mls, 59gms and of density 0.88gm/ml was also obtained from *M. stenopetala* residue after n-hexane extraction. The residue from methanol extraction (cake) weighed 747gms.

4.2 Antibacterial activity.

The results of antibacterial activity was analysed using ANOVA (appendix 1).

4.2.1 *Escherichia coli*

Methanol extracts of *M. oleifera* and *M. stenopetala* showed higher inhibition against *E. coli* at 20% concentration and least inhibition at 2.5%. There was significant difference between the concentrations apart from at 5% and 2.5% where there was no significant difference. There was no significant difference between methanol extracts of *M. oleifera* and *M. stenopetala* against the microbe (Table 2).

Table. 2. Mean area of inhibition on *E.coli* using methanol extract.

Concentration in %	*M. oleifera*	*M. stenopetala*	Mean
20	36.4 a	32.6 a	34.5
10	19.5 b	25.3 b	22.4
40	18.2 b	13.0 c	15.6
5	11.2 c	11.6 c	11.4
2.5	10.4 c	8.6 d	9.5
Mean	19.2	18.2	
LSD	1.786		
CV%	28.61		

Mean followed by the same letter in a column, are not significantly different at p=0.05.

There n-hexane extract showed higher inhibition against *E. coli* at 40% concentration and least inhibition at 5%. There was significant difference between the extracts of *M. oleifera* and *M. stenopetala* against the microbe and *M. stenopetala* exhibited higher inhibition (Table 3)

Table. 3. Mean area of inhibition on *E.coli* using n-hexane extract.

Concentration in %	*M. oleifera*	*M. stenopetala*	Mean
40	36.8 a	26.8 a	31.8
2.5	17.5 c	25.3 a	21.4
20	19.6 b	20.9 b	20.3
10	11.2 d	25.3 a	18.2
5	9.4 e	14.9 c	12.1
Mean	17.2	22.6	
LSD		1.786	
CV%		28.61	

Means followed by the same letter in a column, are not significantly different at p=0.05.

4.2.2 *Salmonella typhii*

The methanol extract of *M. oleifera* and *M. stenopetala* had higher inhibition against the microbe at 5% concentration and least inhibition at 40%. There was significant difference between the extracts of *M. oleifera* and *M. stenopetala* against the microbe and *M. oleifera* exhibited higher inhibition (Table 4).

Table. 4. Mean area of inhibition on *Salmonella typhii* using methanol extract.

Concentration in %	*M. oleifera.*	*M. stenopetala*	Mean
5	67.0 d	26.4 b	46.7
2.5	56.0 c	28.9 b	42.5
10	44.7 b	12.3 a	28.2
20	41.9 d	13.6 c	27.7
40	12.4 e	11.7 c	12.0
Mean	44.4	18.6	
LSD	1.786		
CV%	28.61		

Means followed by same letter in a column are not significantly different at p=0.05.

There n-hexane extract exhibited higher inhibition against the microbe at 2.5% concentration and least inhibition at 40%. There was significant difference between the extracts of *M. oleifera* and *M. stenopetala* against the microbe. *M. stenopetala* showed a higher inhibition. The concentrations showed significant difference a part from 20% and 10% (Table 5).

Table. 5. Mean area of inhibition on *Salmonella typhii* using n-hexane.

Concentration in %	*M. oleifera.*	*M. stenopetala*	Mean
2.5	76.5 a	78.7 a	77.6
5	43.1 b	38.9 b	41.0
20	23.9 c	30.6 c	27.3
10	21.0 d	29.0 c	25.5
40	19.5 e	18.9 d	19.2
Mean	36.9	39.2	
LSD	1.786		
CV%	28.61		

Means followed by same letter in a column are not significantly different at p=0.05.

4.2.3 *Vibrio cholerae*

The methanol extract of *M. oleifera* and *M. stenopetala* had higher inhibition against the microbe at 40% concentration and least inhibition at 2.5%. There was no significant difference between the methanol extracts of *M. oleifera* and *M. stenopetala* against the microbe. There was no significant difference between concentrations 10%, and 2.5% (Table 6).

Table. 6. Mean area of inhibition on *Vibrio cholerae* using methanol extract.

Concentration in %	*M. oleifera.*	*M. stenopetala*	Mean
40	24.5 a	24.5 a	24.5
20	14.9 b	18.8 b	16.9
10	11.7 c	13.2 c	12.5
5	9.2 d	11.7 c	10.5
2.5	8.6 d	8.6 d	8.6
Mean	13.8	15.4	
LSD	1.786		
CV%	28.61		

Means followed by same letter in a column are not significantly different at p = 0.05.

The n-hexane extracts of *M. oleifera* and *M. stenopetala* showed higher inhibition at 2.5% and 5%. There was significant difference at all concentrations. There was significant difference between the n-hexane extracts of *M. oleifera* and *M. stenopetala* against the microbe and *M. stenopetala* exhibited higher inhibition (Table 7).

Table. 7. Mean area of inhibition on *Vibrio cholerae* using n-hexane extract.

Concentration in %	*M. oleifera.*	*M. stenopetala*	Mean
2.5	25.4 b	33.0 a	29.2
5	32.2 a	26.0 b	29.1
10	26.9 b	24.5 b	25.7
20	16.8 c	22.3 c	19.6
40	9.8 d	16.2 d	13.0
Mean	22.2	24.4	
LSD	1.786		
CV%	28.61		

Means followed by same letter in a column are not significantly different at p=0.05.

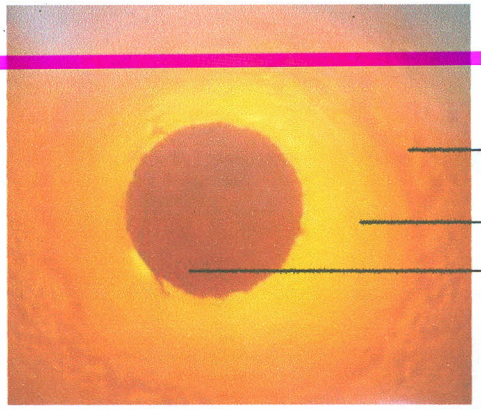


Plate. 3. Zone of inhibition on *S. typhi* at 2.5% *M. oleifera* n-hexane extract

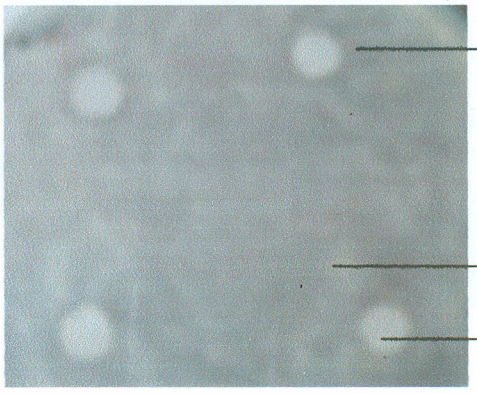


Plate. 4. Zone of inhibition on *V. cholerae* at 40% *M. oleifera* n-hexane extract

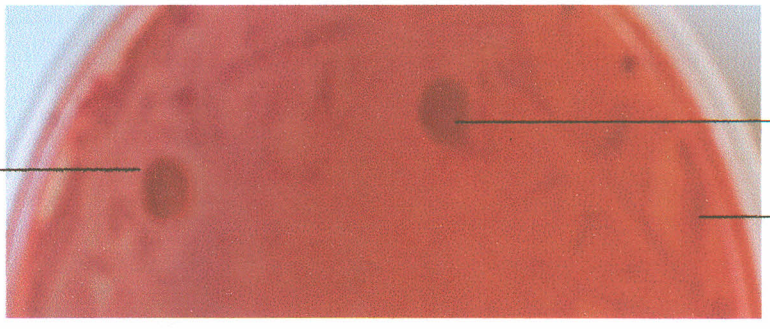


Plate. 5. Zone of inhibition on *E. coli* at 2.5% *M. oleifera* methanol extract

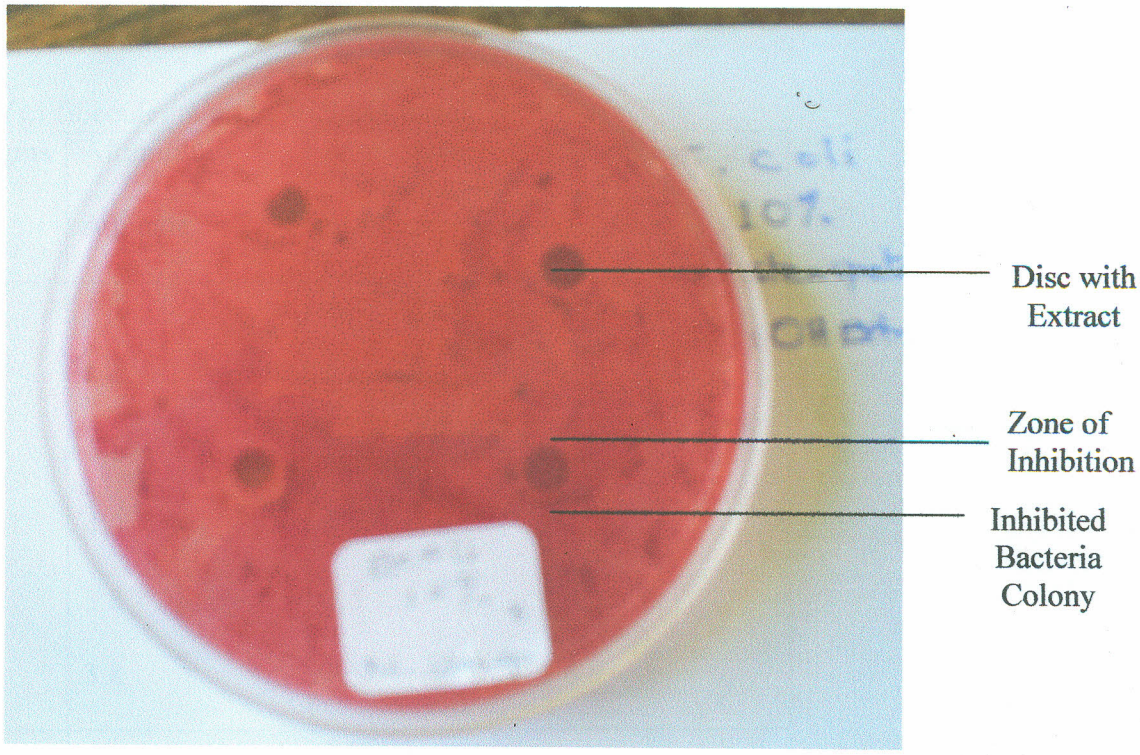


Plate. 6. Zone of inhibition on *E. coli* at 10% *M. stenopetala* methanol extract

4.3 Water clarification

The results of p*H* (appendix 2) and turbidity (appendix 3) were analysed using ANOVA.

4.3.1 p*H* of the water samples.

Table. 8. p*H* of flocculents on L. Victoria water.

Quantity in gms	Aluminium sulphate	*M. oleifera*	*M. stenopetala*
0	7.4	7.4	7.4
0.0625	4.4	7.4	7.1
0.125	4.1	7.5	7.1
0.25	4.0	7.5	7.0
0.5	3.8	7.5	6.9
1.0	3.8	7.5	6.4
LSD		0.0004	
CV%		0.0159	

Table. 9. p*H* of flocculents on Nyamasaria dam water.

Quantity in gms	Aluminium sulphate	*M. oleifera*	*M. stenopetala*
0	6.3	6.3	6.3
0.0625	4.4	7.0	6.9
0.125	3.7	6.9	6.7
0.25	3.6	7.2	7.0
0.5	3.6	7.1	6.9
1.0	3.6	7.1	6.5
LSD		0.0004	
CV%		0.0159	

Table. 10. p*H* of flocculents on River Kibos water.

Quantity in gms	Aluminium sulphate	*M. oleifera*	*M. stenopetala*
0	7.1	7.1	7.1
0.0625	4.2	7.1	7.0
0.125	3.7	6.9	6.7
0.25	3.7	6.9	6.6
0.5	3.8	6.9	5.6
1.0	3.6	7.0	3.5
LSD		0.0004	
CV%		0.0159	

After clarification of the water samples with the flocculents, water clarified using aluminium sulphate had reduced/low p*H* while *M. oleifera* and *M. stenopetala* either increased (Table 9) or maintained (Table 8) p*H* of the water samples. The quantities of the flocculents showed significant difference. *M. oleifera* extract produced the highest p*H* and the lowest quantity (0.0625g) gave the highest p*H* with all the flocculents (Table 8, 9 and 10).

4.3.2 Turbidity of the water samples.

Table. 11. Mean of Turbidity of flocculents on L. Victoria water.

Quantity in gms	Aluminium sulphate	*M. oleifera*	*M. stenopetala*	Mean of concentration
0.0	33.1 a	33.1 a	33.1 a	33.1
0.0625	2.4 c	18.2 b	27.4 a	16.0
0.125	0.7 c	38.0 b	37.9 a	25.5
0.25	0.8 c	47.3 b	52.2 a	33.4
0.5	1.4 c	52.4 b	89.9 a	47.9
1.0	2.1 c	63.5 b	173.9 a	79.9
LSD		0.32		
CV%		1.264		

Means followed by same letter in a row are not significantly different at p=0.05.

Table. 12. Mean of Turbidity of flocculents on Nyamasaria dam water.

Quantity in gms	Aluminium sulphate	*M. oleifera*	*M. stenopetala*	Mean of concentration
0.0	299.7 a	299.7 a	299.7 a	299.7
0.0625	1.1 c	48.6 b	65.6 a	38.4
0.125	1.4 c	129.9 b	103.4 a	78.2
0.25	1.4 c	101.8 b	100.7 a	67.9
0.5	2.5 c	96.4 b	130.1 a	76.3
1.0	2.3 c	84.2 b	216.6 a	101.0
LSD		0.32		
CV%		1.264		

Means followed by same letter in a row are not significantly different at p=0.05.

Table. 13. Mean of Turbidity of flocculents of River Kibos water.

Quantity in gms	Aluminium sulphate	*M. oleifera*	*M. stenopetala*	Mean of concentration
0.0	68.3 a	68.3 a	68.3 a	68.3
0.0625	5.1 c	19.1 b	24.3 a	16.2
0.125	8.3 c	46.6 b	79.3 a	44.7
0.25	6.4 c	44.5 b	86.2 a	45.7
0.5	4.4 c	48.4 b	111.8 a	54.9
1.0	6.7 c	44.9 b	95.0 a	48.9
LSD		0.32		
CV%		1.264		

Means followed by same letter in a row are not significantly different at p=0.05.

The result showed that aluminium sulphate, *M. oleifera* and *M. stenopetala* lowed the turbidity of the water samples after clarification. There was increased turbidity with increase in quantity of the flocculent in the water samples. In some cases the increase in flocculent quantity resulted in increased turbidity beyond the turbidity of the water before clarification (Table 11). The flocculent quantities showed significant difference with the lowest quantity (0.0625g) producing the lowest turbidity. Aluminium sulphate produced the lowest turbidity, followed by *M. oleifera* extract and lastly by *M. stenopetala* extract (Table 11, 12 and 13).

CHAPTER FIVE

DISCUSSION

5.1 Extraction of non-polar and polar components

According to this research finding, *M. oleifera* seeds contain 30% oil and 12% methanol extract. According to Burkill (1966), the seeds yield clear oil to the extent of 22-38.5%. Duke (1987), states that the seed of *M. oleifera* yield 38-40% of oil. Moringa seed yields 30-40% by weight of oil (Tsakins *et al.*, 1999). But according to Folkard and Sutherland (2005), its seeds contain 40% of oil by weight. The difference could be attributed to difference in the environmental and edaphic factors within the area of growth where the plant seeds were obtained. *M. stenopetala* contain 18% oil and 7.0% water soluble active component. Therefore, *M. oleifera* has more oil and water soluble components than *M. stenopetala.* This could be attributed to differences in the species.

5.2 Antibacterial activity

5.2.1 *Escherichia coli*

This research has shown that methanol and n-hexane extracts of both *M. oleifera* and *M. stenopetala* have antibacterial activity against *E. coli*, *S. typhii*, *V. cholera.* The *M. oleifera* and *M. stenopetala* extracts have low efficacy on the microbe (*E. coli*), as shown by low inhibition against the microbe (Table 2). *E. coli* are known to be extremely sensitive to antibiotics such as streptomycin or gentamycin. It is also known to quickly acquire drug resistance (Chandarana *et al.*, 2005). This could be the reason why the extracts of the *Moringa spp*, have shown low efficacy against the microbe.

The inhibition of the microbes reduced with increased extract concentration, an indication that the microbe became more resistant when subjected to extract of higher concentrations. The susceptibility of *E. coli* to the *Moringa spp* extracts increased with decrease in extract concentration upto 20% (Table 2) and susceptibility reduced below 20% extact concentration. Another study showed that the defatted seed contains 8-10% of the antibacterial agent (Eilert, *et al.*, 1981). A study conducted in India, in which crude ethanol extract of *M. oleifera* were tested against *E. coli*, *S. typhii*, *V. cholera*, *Shigella dysentriae* and *Pseudomonas pyocyaneus*, showed activity against *E. coli* only (Shekhar, *et al.*, 2000). This was in variance with our findings which showed efficacy in *E. coli*, *S. typhii* and *V. cholera*. A research conducted in Thailand showed that *M. oleifera* crude extract had no activity against *E. coli, showing* variance with our findings. However, the purified dichloromethane extract and isolated parts from column chromatography showed antibacterial activity against *E. coli* (Khesorn, 2009).

5.2.2 *Salmonella typhii*

This research has established that the seed extracts of the *M. oleifera* and *M. stenopetala* have antibiotic activity against *S. typhii*. Both the methanol and n-hexane extracts have antibiotic activity on the microbe (Table 4 and Table 5). *S. typhii* was inhibited at lower concentrations making methanol extract more cost effective to use as antibiotic against the microbe. The methanol extract can be used at low concentrations in purification to prevent the spread of salmonellosis (Swanson *et al.* 2007). *M. oleifera* methanol extract exhibited higher microbial inhibition against the microbe compared to *M. stenopetala* (Table 4). This is advantageous since it is available in Kenya and within the study area in

particular. The oil produced can be used for cooking (Folkard and Sutherland, 2005) and is also antibiotic in nature (Melaku and Yohanes, 1988). When used for cooking, it sterilizes food, and serves as medicine against typhoid since *S. typhii* is spread through contaminated food and lives in the intestinal tracts of man. The antibiotic and anti-ulceration nature of the *Moringa spp* makes the oil ideal for preparing food since the oil form a thin film over the intestinal wall when eaten thus reducing or preventing the pathogen (by inhibition) from penetrating the walls (Caceres and Lopez, 1991; Caceres, *et al.*, 1991; Nwosu and Okafor, 1995). It is evident from other studies that *Moringa spp* have shown antibiotic activities against *Helicobacter pyliri* (Harristoy *et al.*, 2005). Another study has also shown that, the oil from *M. oleifera* seed has antibacterial activity (Badgett, 1964). Other studies indicated that the *Moringa spp* produce gum that is found to be anti-typhoid in their activity (Fuglie, 1999). Our findings confirmed the same. However, a study conducted in India showed that the crude ethanol extract of *M. oleifera* seed showed no activity against *S. typhii* (Shekhar, *et al.*, 2000). This was in variance with our results. The antibacterial activity of the plant has been demonstrated against both gram-negative and gram-positive bacteria, this is in agreement with our findings. (Siddhuraju and Becker, 2003; Vaghasiya and Chanda, 2007; Mashiar *et al.*, 2009).

5.2.3 *Vibrio cholerae*

This study has shown that the methanol and n-hexane extracts had antibacterial activity against *V. cholerae.* The methanol and n-hexane extracts had lowest inhibition on *V. cholerae* compared to *E. coli* and *S. typhii*. This is an indication that *V. cholerae* had higher resistance to the extracts (Table 6 and 7). The n-hexane extract showed it had

higher inhibition than methanol extract for both the *Moringa spp* (Table 6 and 7). The n-hexane extract inhibition against *V. cholerae*, increased at lower concentrations. This indicated that *V. cholerae* was resistant to the extracts at higher concentration. Since *V. cholerae* produces an enterotoxin whose action on the mucosal epithelium lining of the small intestine is responsible for the characteristic massive diarrhea (Ryan and Ray, 2004), when the oil is used for cooking it serves as food sterilizer and acts against the pathogen reducing the spread of the disease. This could be made possible as the oil forms a thin layer on the epithelium causing inhibitive effects on the microbe. Cooking oil, keeps the food free from disease causing micro-organism i.e. *S. typhii, V. cholerae* and *E. coli* as indicated by the oils antibacterial ability. The oil also serves as medicine. The soap made from the oil also have antiseptic properties, thus it is of medicinal value against the skin infections. From these research findings, Moringa plant extracts were found to be effective against *S. typhii* than *E. coli* and *V. cholerae*. The extract could be used or medicine developed from it to treat the diseases in humans since Moringa plant products have no known health risks on humans (Jahn, 1981; Bergeret *et al.*, 1984: Sutherland *et al.*, 1990). Infact, it has several uses, in water purification (Madsen *et al.*, 1987; Yongbai, 2005), sedimentation (Yongbai, 2005), antiosis and even reduction of *Schistosoma cercarie* titer (Olsen, 1987).

Moringa plant preparations have antibiotic and this agrees with our findings (Nwosu and Okafor, 1995; Harristoy *et al.*, 2005), this antitrypanosomal (Mekonnen *et al.*, 1999), antihelminths (Fuglie, 1999; 2001), hypotesive (Jadhav, 2000), antispasmodic (Faizi, 1998), antiulcer (Caceres and Lopez, 1991), anti-inflammatory (Jadhav, 2000),

hypocholesterolemic (Ghasi *et al.*, 2000) and hypoglycemic activities (Kar and Chounddhary, 1999), HIV-AIDs management (Prazuk, 1993), anti-Herpes simplex virus type 1 (Lipipun *et al.*, 2003). However, a study conducted in India using crude ethanol extract of *M. oleifera* showed no activity against *V. cholerae* (Shekhar, *et al.*, 2000) in variance with our findings.

5.3 Water clarification

According to results of the research, after aluminium sulphate was applied to the water samples, the p*H* drastically dropped, making the clarified water acidic. Lime or soda ash is normally added by the municipal water works to raise the p*H*. This is an additional cost that the poor people in the rural area cannot afford. It also results to water hardness, making the water less tasty for drinking and costly to use. Such acidic water are health hazard to humans since it leads or even accelerates development of stomach ulcers. *Moringa spp*, however have maintained or adjusted the p*H* to the recommended standards (Table 8 and 9). The study showed that *M. oleifera* produced the highest p*H*, followed by *M. stenopetala* and aluminium sulphate produced the lowest p*H*. The *Moringa spp* maintained/adjusted the p*H* of the clarified water to the recommended water quality standards, i.e. KIWASCO; 6.5-7.6, Kenya Bureau of Standards; 6.5-8.5; WHO; 6.5-8.5 and EU/USA; 6.5-9.5 (The Handbook-DR/2400), while aluminium sulphate lowed the p*H* drastically (Table 8, 9 and 10).

After clarification with aluminium sulphate, *M. oleifera* and *M. stenopetala* extracts, lower turbidity was obtained for water samples from Nyamasaria dam, Kibos River and

L. Victoria (Table 11, 12 and 13). The turbidity of the water samples after clarification without filtration, was however higher than the recommended water quality operating standards for turbidity, i.e. KIWASCO; 5 NTU, Kenya Bureau of Standards; 5 NTU; WHO; 5 NTU and EU/USA; 5 NTU (The Handbook-DR/2400). When higher concentrations of methanol extract of *M. oleifera, M. stenopetala* and aluminium sulphate were used in clarification of the waters, even higher turbidities were realized. The increased turbidity was as a result of free positively charged molecules of the flocculents repelling, leading to the flocs floating or suspended in the water (Sutherland *et al.,* 1990; Sutherland *et al.,* 1994). Such floating flocs could be filtered to achieve lower turbidity. The research indicated that without filtration, the methanol extract of *M. oleifera* and *M. stenopetala* had water clarifying ability lower than that of aluminium sulphate in turbidity reduction while *M. oleifera* and *M. stenopetala* extracts were better than aluminium sulphate in p*H* adjustment.

Studies in the West countries such as America have shown that the best known use for *Moringa spp* is the use of seeds powdered to flocculate and clarify drinking water (Berger *et al.,* 1984). This compare with our findings. Other studies have shown that unpurified crushed seeds were used in water clarification, but it had several disadvantages since as water takes long time in the distribution system, the organic material in the seed become septic, leading to growth of microorganisms and the formation of harmful by-products after chlorination. This has made it necessary to research on use of the extracts as water clarifier. A research study showed that purified coagulant from the extract of *M. oleifera* showed both flocculating and antibacterial effects with samples of water of high

turbidity. *M. oleifera* was found to be more effective for bacterial removal from water than *M. stenopetala* (Madsen, *et al.*, 1987). Another study has shown that, the extract showed similar coagulation activity as aluminium sulphate (Ghebremichael, 2005). Research done at the University of Daresalam Tanzania showed that Moringa plant seed extracts had coagulation and antimicrobial activity (Suerez *et al.*, 2003). This agrees with our findings.

A comparative study was done using *M. oleifera*, aluminium sulphate and a mixture of *M. oleifera* and aluminium sulphate. The result obtained indicated that water clarified with *M. oleifera* and aluminium sulphate recorded turbidities less than the required standards of 5 NTU, while a mixture *M. oleifera* and aluminium sulphate recorded the least turbidity (Liew *et. al.*, 2006). This shows that *M. oleifera* had high efficacy in water clarification. A comparative study of *M. oleifera* seed powder and aluminium sulphate gave a turbidity and p*H* within the acceptable WHO range (Francis and Amos, 2009). The above studies were confirmed by our findings.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

M. oleifera and *M. stenopetala* have antibacterial activity against *S. typhii*, *V. cholerae* and *E. coli*.

M. oleifera and *M. stenopetala*, have lower water clarifying ability than aluminium sulphate.

RECOMMENDATIONS

The rural population should use *Moringa spp* particularly *M. oleifera* in water purification due to its better antibacterial activity.

The rural population should use *Moringa spp* particularly *M. oleifera* in water clarification due to its ability to reduce turbidity and maintain p*H* of water.

The rural population should be encouraged to plant at least one Moringa tree per household to be used for water clarification and purification.

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